HAZARDOUS AIR POLLUTANT EMISSIONS FROM THE EXTERNAL COMBUSTION OF HYDROCARBON GASEOUS FUELS CAN BE PREDICTED!

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INTRODUCTION

Passage of the 1990 Amendments to the Clean Air Act made it clear that maximum available control technology ("MACT") regulations on the emission of hazardous air pollutants ("HAPs") from process heaters and industrial boilers, used extensively in the petroleum and petrochemical industries, would be promulgated under congressional mandate by the U. S. Environmental Protection Agency in the year 2000. Unfortunately, it had also become clear that *understanding*, the "good science" upon which we aspire to base sensible regulations, was simply non-existent and, further, that the little field data then extant was severely flawed. To amend those deficiencies, a 4-year $7-million fundamental attack on the origin and fate of trace toxic emissions in the external combustion of gaseous hydrocarbon fuels has been conducted by a government-university-industry collaboration[1] that has been, by all accounts, one of the most successful ever.

A number of challenges were encountered during the course of the project. Perhaps foremost among them and exemplary of the remarkable strength of this collaboration, was the need not only to expand the capabilities of the Sandia National Laboratories, Livermore, Combustion Research Facility Burner Engineering Research Laboratory (BERL) by the addition of 4-component fuel mixing capability (formerly natural gas only); and bunkering and delivery capability for the various gases to be employed in the full-scale burner trials (hydrogen, propane, propylene, ethylene); but also, at the cost of over a quarter of a million dollars that was quickly raised by the CRADA-signatories, to convert the former BERL flame laboratory into a process heater laboratory by the addition of a convection section simulator.

This allowed the project successfully to reproduce at full-scale the generally very low normal-operation gaseous-combustion emissions that are observed in the field, as well as higher hypothetical extreme failure-mode emission levels of aldehydes, volatile organic compounds, polycyclic aromatic hydrocarbons and total organics fully comparable to the highest levels reported in field tests, however sparse and unreliable they might be, observed under extreme combustion conditions that would neither be tolerated nor observed in actual field operations but that, nevertheless, proved to be required to generate toxic emissions of any significance.

Through the rigorous and highly reliable measurements of regulatory development quality, as guaranteed by the project's exemplary Quality Assurance Project Plan that was produced in cooperation with U. S. Environmental Protection Agency experts, carried out during the full-scale burner trials conducted at the Sandia National Laboratories, Livermore, Combustion Research Facility Burner Engineering Research Laboratory, a great deal has been learned, in far more detail and under many more conditions, about the normal and hypothetical extreme limiting emissions from full-scale burners than was heretofore known. However, this paper discusses only the findings that most pertinently address the question, "Can we predict HAP emissions based on fuel composition?"

HAP EMISSIONS UNIFORMLY LOW

In this program we saw over and over again that the nature of the gaseous hydrocarbon fuel mixture doesn't make much difference, neither in the total toxic emissions nor in the individual species levels. This observation includes natural gas which is itself, after all, merely just another hydrocarbon mixture; *i.e.*, there is no reason to distinguish "refinery fuel gas" from "natural gas." Figures 1 and 2 illustrate this equivalency on a speciated basis, there being only small and statistically insignificant differences in the individual species emissions.

Almost stochastic in nature, the individual species levels are uniformly exceedingly low, seemingly less dependent upon physics and chemistry and more dependent upon the vagaries of the sophisticated sampling methods and precise analytical techniques that are required to detect them at all in the minute concentrations in which they appear in the combustion products. In Figures 3-6, these facts are illustrated by the mass emission of total hydrocarbons. Except for an operationally unrealistic super-aerated (450% stoichiometric air) case, we see that there is no significant effect of heating value, combustion zone stoichiometry, propylene or ethylene spikes, nor hydrogen content in the gaseous hydrocarbon fuel mixtures.

The heating value variation was achieved at the constant base case 16% hydrogen content simply by increasing the proportion of propane in the hydrogen, natural gas, propane mixture. The field-operational typical ±15% theoretical air variation around the base case 125% was extended substoichiometrically in the combustion zone to 50% or one-half of the air theoretically required for complete combustion with overfire air added to simulate a leaky furnace while still maintaining the base case 125% theoretical air in the stack. The theoretical air variation was extended super-stoichiometrically in the combustion zone to 450% or four-and-one-half times the air theoretically required for complete combustion simply by increasing the air delivery to the

[1] Petroleum Environmental Research Forum Project Number 92-19 sanctioned under a Stevenson-Wydler (15 USC 3710) Cooperative Research and Development Agreement

burner. The effect of spikes of ethylene and propylene on the emissions of the base case 16% hydrogen, 1050 Btu/scf fuel mixture was tested by utilizing the four component mixing capability that was added by this program to the Sandia National Laboratories, Livermore, California, Combustion Research Facility's Burner Engineering Research Laboratory, while the effect of hydrogen variation on emissions from the 1050 Btu/scf base case was tested simply by compensating adjustments to the natural gas and propane fractions in the fuel mixture.

The absence of systematic variability in the trace emission of toxic byproducts in gaseous external combustion is strikingly illustrated in Figures 7 and 8. We see that the reproducibility of the reference regulatory base cases ("A1" was a 1050 Btu/scf mixture of 16% hydrogen, natural gas, and propane while "A4" was 1050 Btu/scf natural gas) remained good throughout all of the conventional diffusion flame burner ("CDFB") trials in test sequences A, B and C. While test sequence A spanned a broad range of fuel compositions and operating conditions around the normal-operation base cases A1 and A4, we saw no systematic variation in emissions; all emissions remained exceedingly low and the small differences were well within the typical bounds of experimental variability.

Worried that, even in the sequence B "failure mode" tests, we were not able to reproduce polycyclic organic hydrocarbon ("PAH") emissions as high as those reported in some field tests, however unreliable those field tests may have been, we redoubled our efforts to fail combustion and, in the sequence C "super-failure mode" trials, we were rewarded with stack emissions up to 5E-6 lb-PAH/mmBtu (*e.g.*, B13' in Figures 7 and 8), fully as high as any in the "real world" field data base. These high emissions are often attributed, but without much definition and no detailed understanding, to "gross mixing failures." We saw in this program, as illustrated in Figures 7 and 8, that to generate high stack emissions from gaseous hydrocarbon mixtures in external combustion, fuel-air mixing failures of the grossest kind are indeed required, egregious hypothetical extreme combustion conditions that would hardly be tolerated nor permitted to persist in any well-run plant.

HIGH VELOCITY JET MIXING ACCOUNTS FOR LOW TOXICS

The strong mixing potential of sonic jets is well known. In the case of the multiple, small reacting jets of the conventional diffusion flame burner, surrounded as they are under normal conditions with an excess supply of air, why would we not expect very low toxic emissions?

Early in the program, we hypothesized that the hot, rich combustion regions that are necessarily present in a diffusion flame ought to be prolific generators of toxics. The early stirred-reactor, plug-flow computations carried out by Lawrence Livermore National Laboratory supported the hypothesis, while later the laboratory flame measurements carried out at the UCLA Chemical Engineering Laboratory, as well as the research furnace experiments carried out at the Sandia National Laboratories, Livermore, Combustion Research Facility, confirmed it. The Lawrence Livermore calculations also suggested that, in the presence of excess air, the toxics that are necessarily profusely-generated in the rich zone would subsequently quickly be consumed to near-extinction, a prediction that we have now seen borne out time and time again in the full-scale burner trials carried out at the Sandia Combustion Research Facility's Burner Engineering Research Laboratory.

Most significant are the results of Sandia's application of a two-stage Lagrangian jet model to a typical conventional diffusion flame burner jet. Based upon the observed flame structure of the conventional burner, the jet model was applied twice: first to the individual jet flames that emerge from the burner tips inside the quarl and again for the merged jet exiting the quarl; thereafter, when mixing is completed, a plug-flow reactor model is utilized to represent the remaining flow to the furnace exit. To give confidence in the results, it may be observed that the model predicted a final CO level of 2 ppm, consistent with the actually measured level below the detection limit of 5 ppm, and a final NOx concentration of 106 ppm, compared with the measured value of 118 ppm. The jet model predicts that the air toxic species should be produced to significant levels within the in-quarl flames but should be consumed well within the substoichiometric regime, both just as we have seen in the full-scale trials.

The Lagrangian jet model confirms and illustrates the expected behavior. Initially, where the reactions are just beginning, there is nothing but the original fuel reactants and oxidant in abundance. As the reactants and oxidant begin to mix, the reacting part of the "reacting jet" begins, too, and the reaction products begin to appear. Then as more and more air is mixed into the jet, with theoretical air % increasing but still well within the substoichiometric regime, the reaction products peak but then are rapidly consumed even before the mixture reaches stoichiometric.

The prediction confirmed that toxic species, manufactured in abundance in the hot, rich diffusive regime, are subsequently consumed in the high-mixing-potential jet well before it reaches even stoichiometric conditions, is, of course, extremely significant, not only with regard to its implication upon the robustness of practical combustion systems in the field but also with respect to the predictability of HAP emissions based on fuel composition.

Moreover, it is perhaps remarkable to note that, in the super-failure mode full-scale trials carried out at the Sandia National Laboratories, Livermore, Combustion Research Facility's Burner Engineering Research Laboratory, it was not until severely substoichiometric conditions (stoichiometric ratio below 0.80) were achieved in the combustion zone and maintained right

through and out the stack to the atmosphere, and just as predicted by the Lagrangian jet model, that high levels of toxics emerged.

This goes a long way toward explaining why the conventional diffusion flame burner, composed as it is of burner tips out of which emerge high-mixing-potential jets surrounded by an abundant supply of oxidant, simply has to be a low toxics burner.

CONCLUSION

Jet-mixed gaseous hydrocarbon diffusion flames, such as those produced by the burners that are typically used in petroleum industry process heaters and industrial boilers, result in a combustion process that is extremely robust, producing predictable, exceedingly low emissions of hazardous air pollutants even when subjected to extreme mixing failures. Readers who are interested in learning more about this interesting subject are referred to the papers published in learned journals that emerged from this program, many of which are listed in the references.

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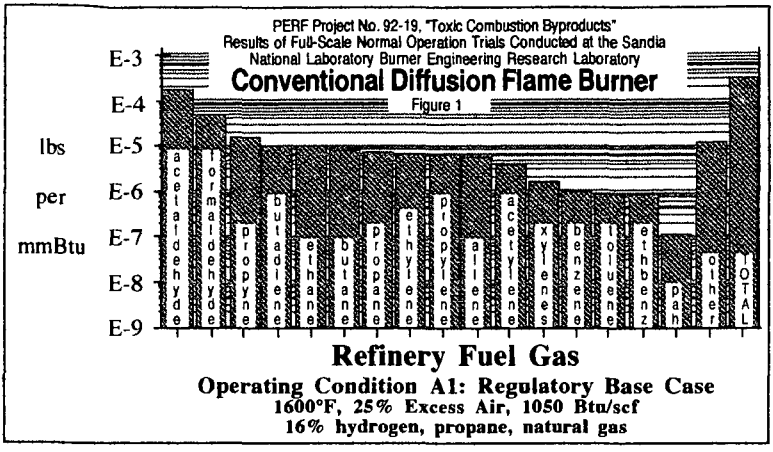
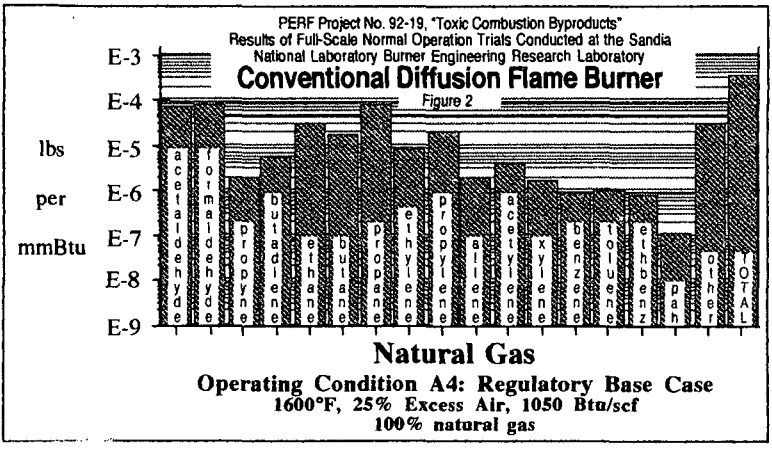
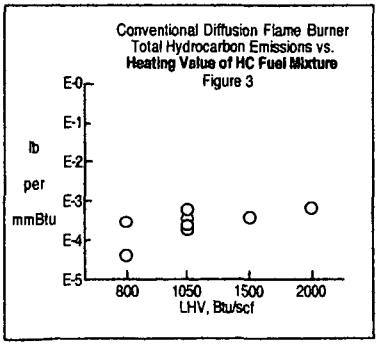
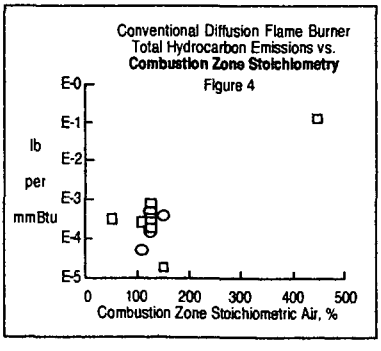
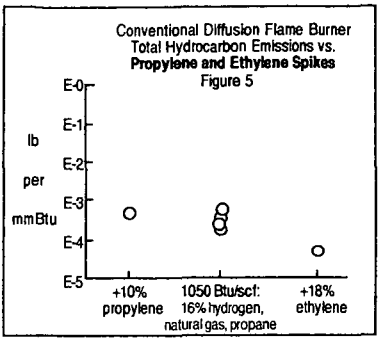
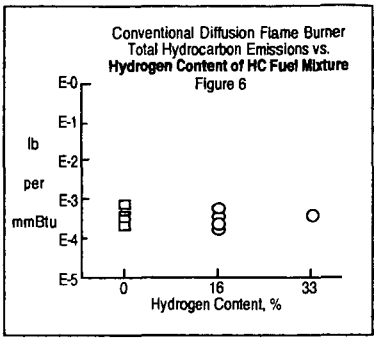

PERF Project No. 92-19, "Toxic Combustion Byproducts"
Results of Full-Scale Normal Operation Trials Conducted at the Sandia
National Laboratory Burner Engineering Research Laboratory
Conventional Diffusion Flame Burner
Figure 1
lbs per mmBtu
E-3
E-4
E-5
E-6
E-7
E-8
E-9
acetaldehyde
formaldehyde
propyne
butadiene
ethane
butane
propane
ethylene
propylene
allene
acetylene
xylenes
benzene
toluene
ethbenz
pah
other
TOTAL
Refinery Fuel Gas
Operating Condition A1: Regulatory Base Case
1600°F, 25% Excess Air, 1050 Btu/scf
16% hydrogen, propane, natural gas


PERF Project No. 92-19, "Toxic Combustion Byproducts"
Results of Full-Scale Normal Operation Trials Conducted at the Sandia
National Laboratory Burner Engineering Research Laboratory
Conventional Diffusion Flame Burner
Figure 2
lbs per mmBtu
E-3
E-4
E-5
E-6
E-7
E-8
E-9
acetaldehyde
formaldehyde
propyne
butadiene
ethane
butane
propane
ethylene
propylene
allene
acetylene
xylene
benzene
toluene
ethbenz
pah
other
TOTAL
Natural Gas
Operating Condition A4: Regulatory Base Case
1600°F, 25% Excess Air, 1050 Btu/scf
100% natural gas


Conventional Diffusion Flame Burner
Total Hydrocarbon Emissions vs.
Heating Value of HC Fuel Mixture
Figure 3
lb per mmBtu
E-0
E-1
E-2
E-3
E-4
E-5
800
1050
1500
2000
LHV, Btu/scf


Conventional Diffusion Flame Burner
Total Hydrocarbon Emissions vs.
Combustion Zone Stoichiometry
Figure 4
lb per mmBtu
E-0
E-1
E-2
E-3
E-4
E-5
0
100
200
300
400
500
Combustion Zone Stoichiometric Air, %





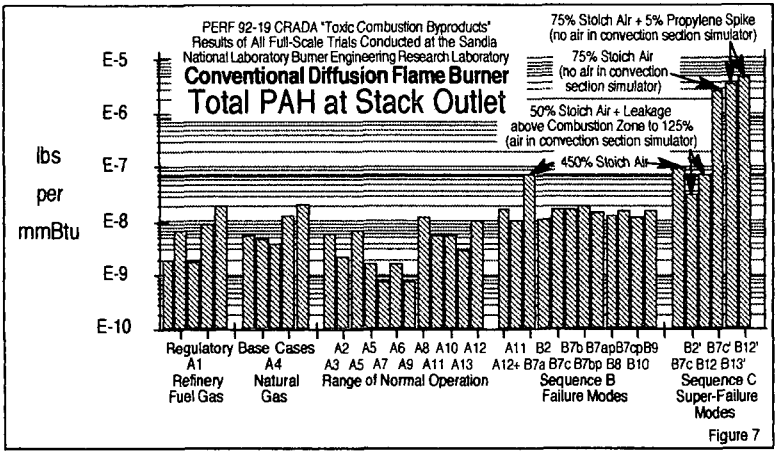


Figure 7

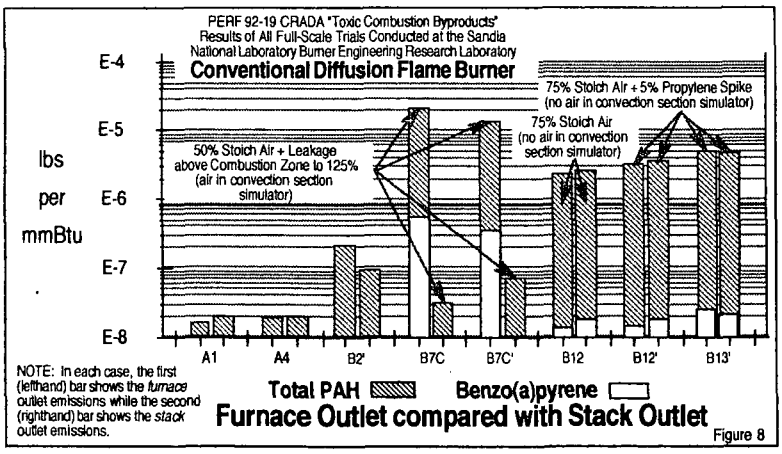


Figure 8

IMPACT OF OPERATING CONDITIONS AND FUEL COMPOSITION ON VEHICLE EMISSIONS

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INTRODUCTION

Control of exhaust hydrocarbon (HC) emissions is an important feature in the design of motor vehicles. Both the Federal Government (1990 Clean Air Act Amendments[1]) and the State of California[1] instituted 25% decreases in the permitted HC emission levels in the early 1990's. The California regulations required an additional reduction in fleet-averaged, new automobile HC emissions from 0.25 gm/mile NMHC in 1993 to 0.062 gm/mile NMOG in 2003.

The Federal Clean Air Act defines specific toxic exhaust species, which may be subject to future control (e.g. benzene, butadiene, formaldehyde, acetaldehyde). California will require that the reactivity of the emissions for smog formation (the Ozone Forming Potential), rather than total HC mass, form the basis of the regulations. To meet these species-specific requirements, concentrations of individual HC species in the exhaust must be measured, and the chemistry of the emissions process must be understood better.

The bulk of the FTP (Federal Test Procedure) emissions from current vehicles occur early in either a cold start or a warm restart before the catalytic converter reaches its minimum operating temperature.[2] Therefore, these start-up emissions are engine-out emissions, unaffected by the catalyst. The effects of fuel composition and engine operating conditions on engine-out HC emissions (both total and individual species) are the subjects of this presentation. Initially, sources of unburned hydrocarbon emissions will be presented. The effects of fuel composition, engine operating conditions, and air/fuel mixing on these emissions will then be discussed in the context of the ability to accurately predict HC emissions from vehicles.

SOURCES OF EXHAUST HCS

Oxidation in the combustion chamber is generally an efficient process with little HC escaping combustion during fuel-lean operation. Nonetheless, unburned fuel and fuel-derived combustion products are exhausted from engines, and this section examines major sources of these emissions.[3,4]

CREVICE VOLUMES - For all operating conditions, a principal source of HC emissions is storage of unburned fuel in crevice volumes around the piston rings (~5-7% of the intake charge). Because the entrances are narrow, the flame cannot enter these crevices, leaving the fuel in them unburned. This fuel leaves the crevices during the expansion stroke, and a large fraction of the stored HC (~50-90% depending upon operating conditions and fuel composition) is converted to CO or CO_2 in the hot burned gases within the cylinder or in the exhaust system. Thus, late cycle burn up of stored HCs affects both the total HC emissions and the concentrations of important partial combustion products such as olefins, butadiene, and benzene.

BULK GAS QUENCHING - When an engine runs fuel rich, the contributions of methane and acetylene in the exhaust rise rapidly.[5,6] These species are present in the core gas within the cylinder after flame propagation is complete. This occurs because the low level of oxygen in the post-flame gas slows the conversion of these intermediate combustion products to CO and CO_2. Incomplete combustion during marginal operation (i.e. very fuel lean or high exhaust gas recirculation) can also increase emissions of all HC species because the flame speed is too slow to complete fuel consumption within the cylinder during the power stroke. These sources are unrelated to crevice storage but are affected by late-cycle burn-up.

WALL WETTING BY FUEL - Another important exhaust HC source arises when liquid gasoline enters the combustion chamber and strikes its walls during cold start of a port-fuel-injected (PFI) engine, producing a fuel film which does not evaporate and burn completely during flame passage.[7] This can increase the total HC emissions early in a cold start relative to the emissions observed with prevaporized gasoline fuel, which minimizes wall wetting (see Figure 1). As the engine warms, the HC emissions from the two fueling techniques approach one another. Wall wetting increases the contribution of lower volatility species such as aromatics to the exhaust emissions. The HC emissions from this source are influenced by the design of the port, combustion chamber, and

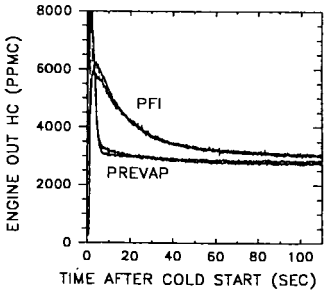

Figure 1. Total HC emissions vs time after cold start for PFI and prevaporized starts in a V8 engine.

injection system. Wall wetting does not contribute appreciably to HC emissions during warmed-up operation because fuel evaporation occurs more rapidly.

EXPERIMENT

To assess the effect of fuel composition on emissions of individual HC species, we have carried out a series of experiments in which the same single-cylinder engine was run at four operating conditions using single-component hydrocarbon fuels. The fuels tested span most classifications found in gasoline and several alternative hydrocarbon fuels: alkanes;[8] naphthenes;[9] olefins;[10] and aromatics.[8, 9, 10] Emissions from one gasoline and selected synthetic fuel mixtures were also measured[9, 10] providing information about interactions between fuel components.

A 475 cm^3, port-fuel-injected, single-cylinder engine with 9:1 compression ratio was used in these experiments. The head and piston have geometries typical of modern multi-cylinder engines. The engine was run at four steady-state conditions. The baseline condition was 10% fuel-lean, 1500 rpm, mid-load, optimum spark timing, and 90°C coolant temperature. Fuel injection took place onto a closed intake valve. Additional conditions studied were: 2500 rpm; retarded spark; or 15% fuel-rich with the other parameters as defined for baseline.

Total emissions were measured by a heated, flame ionization detector (HFID) connected to the exhaust pipe by a heated sample line. Samples were also taken for gas chromatographic (GC) analysis of HCs.[9] With the exception of benzaldehyde, MTBE, and methacrolein, oxygenated organics were not quantified. However, the measured hydrocarbon species account for approximately 90% of the organic emissions and atmospheric reactivity.

FUEL STRUCTURE EFFECTS ON TOTAL HC EMISSIONS

The total HC emissions vary with fuel structure as illustrated in Figure 2 for nine fuels even though nearly the same amount of fuel mass is stored within crevices for all fuels. The total exhaust HC emissions increase from 320 $ppmC_1$ [$ppmC_1 = \Sigma_i(ppm_i x \#carbons_i)$ for all exhaust species i] for ethylene fuel to ~2200 $ppmC_1$ for an aromatic blend (80% xylenes/ 20% ethylbenzene). Only a small portion of this increase arises because the carbon content of the inlet charge is 20% larger for the blend than for ethylene fuel as a result of the different H/C ratio. Similar trends in total HC emissions with fuel structure are observed for the other engine operating conditions.[8, 9, 10] Total HC emissions from olefinic fuels are lower than those from their paraffinic analogs in all cases.[10] The results in Figure 2 demonstrate that, during the exhaust stroke, burn up of fuel stored in crevices is affected by fuel structure and exerts a large influence on HC emissions.

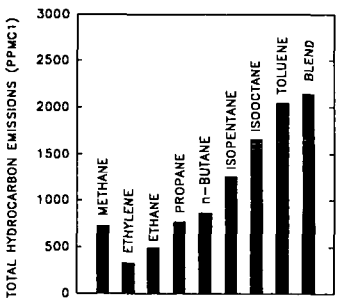

Figure 2. Effect of fuel structure on total HC emissions for nine fuels at baseline condition.

Table I. Exhaust composition as a percentage of total HC emissions for isooctane and toluene fuels.

	ISOOCTANE		TOLUENE	
exhaust species	base (%)	2500 (%)	base (%)	2500 (%)
methane	1.7	4.8	0.8	1.3
ethylene	3.5	9.3	0.8	3.0
propylene	8.9	13.7		
butadiene	0.5	0.9	0.3	n.a.
isobutene	22.9	25.3		
isooctane	46.5	21.7		
benzene	0.3	0.6	5.9	11.0
benz-aldehyde			5.0	7.3
toluene			80.0	63.9
total HC ($ppmC_1$)	2006	1207	2040	1320

Recent experiments[11] in which HC oxidation in the exhaust system was stopped by introducing a cold quench gas near the exhaust valve show that the fuel structure effect on total emissions occurs within the engine cylinder. Continuing oxidation in the exhaust system influences the individual HC species concentrations significantly and reduces the total emissions by ~40% for all fuels at baseline condition. These results illustrate the importance of burn up late in the engine cycle and in the exhaust system on HC emissions.

EXHAUST HC SPECIES

PARAFFINIC FUELS - Table I presents distributions of selected HC species (as a percentage of the total HC emissions) and the total HC emissions measured by the HFID for isooctane (2,2,4 - trimethylpentane) fuel operated at two steady-state conditions (base and 2500 rpm).[9] Although the concentrations of specific species depend on the fuel structure,

the trends shown by isooctane fuel are typical of other straight chain and branched chain alkanes.[8, 9, 10]

Increasing the engine speed results in a decrease in the percentage of unburned isooctane fuel (47% to 22%) in the exhaust, while the sum of the olefinic combustion products increases (43% to 56%). The total exhaust HC concentration decreases indicating an increase in burn-up of stored HCs, primarily because of higher exhaust temperatures. Similar trends with speed have been observed using a multi-cylinder engine[12] and on-road vehicles.[13]

The single cylinder engine results in Table I are similar to the species distributions obtained from two multi-cylinder engine experiments using pure isooctane fuel. In a 2.3 L 4-cylinder engine at baseline condition, [6] the contributions of the major HC species were: methane (1.5%); ethylene (6.3%); propylene (13.5%); isobutene (28%); isooctane (48%). Shore et al[12] determined distributions from a Fiat 4-cylinder engine at 2400 rpm: methane (7%); ethylene (11.1%); propylene (12.7%); isobutene (20.5%); isooctane (23.6%). The agreement of the data in refs. 6 and 12 with the base and 2500 rpm results in Table I, respectively, is reasonably good. This indicates that HC species distributions from very different engines operated under similar conditions can resemble one another, although they are not identical

Alkene emissions from alkane combustion can be explained qualitatively by known high temperature reactions of alkyl radicals (see ref. [10]), formed by H atom abstraction from fuel species. The primary reactions of these radicals are to break a C-C bond one removed from the free radical site (β C-C bond scission[14]) or a C-H bond, both forming alkenes.

AROMATIC FUELS - Table I also presents data for toluene fuel.[8, 10] For toluene as well as for the aromatic blend,[9] unburned fuel constitutes a much larger portion of the exhaust HC than for isooctane fuel and is the predominant species under all operating conditions. In addition, these experiments show that benzene, a toxic species, constitutes an appreciable fraction of the total emissions (6-11%) in contrast to isooctane fuel. This confirms that dealkylation of substituted benzenes is a significant source of benzene emission.

Table II Exhaust composition as a percentage of the total HC emissions for cyclohexane and 1-hexene fuels.

	c-HEXANE		1-HEXENE	
exhaust species	base (%)	2500 (%)	base (%)	2500 (%)
methane	1.3	4.8	1.6	3.1
ethylene	23.3	31.7	24.1	37.5
propylene	5.2	6.0	11.0	12.4
butadiene	12.1	11.7	9.5	10.8
benzene	4.8	6.5	0.6	1.3
c-hexane	30.0	11.7		
c-hexene	6.2	4.0	0.5	0.5
1-hexene			36.9	12.5
total HC ($ppmC_1$)	1190	575	1110	400

OLEFINIC AND CYCLIC ALKANE FUELS - Table II presents selected species measured at base condition and at 2500 RPM for 1-hexene[10] and for cyclohexane[9] fuels. These types of fuels, which have no branching groups, produce large quantities of ethylene, (which is very reactive in the atmosphere), again by β C-C bond scission reactions.[9,10] In addition, they emit more 1,3-butadiene than any of the other fuels tested. It is interesting to note that cyclohexane fuel produces appreciable quantities of benzene in the exhaust. The concentration of benzene is approximately one half of that emitted by toluene fuel and 10x more than is present in the exhaust from isooctane.

ATMOSPHERIC REACTIVITY - The reactivity of individual exhaust HC species for forming photochemical smog varies widely. Olefins and highly alkylated aromatics can have reactivities 5-10 times larger than paraffinic fuels.[15] As discussed above, the distribution of HC species in the exhaust changes as the fuel type and engine operating conditions change. Thus, the atmospheric reactivity of the exhaust gas, which is an important factor in the California regulations, can change significantly, and engine operating strategies different from those used in meeting total HC standards may be required. Fuel structure and operating parameter effects on reactivity are complicated as has been discussed elsewhere.[16]

TOXIC EMISSIONS - The brief descriptions of the species emissions for selected examples of fuel components encountered in gasoline have demonstrated that each class of fuel produces a very characteristic distribution of species in the exhaust gas. Thus, control of the emission of specific species such as the toxic compounds defined in the Clean Air Act Amendments (e. g. benzene and 1,3-butadiene) can be achieved only after identifying the likely precursors of these emissions in gasoline. Benzene is formed in substantial quantity by toluene (5% of the total emissions at baseline condition). The xylene-ethylbenzene blend produces benzene emissions at a lower level (2.5% at baseline), and also generates appreciable toluene (3.7% at baseline).[9] Thus, these results show that reduction of benzene emissions can be achieved by reduction of aromatics in the fuel but that all alkyl-substituted benzene fuels do not produce the same amount of benzene in the exhaust.

1,3-Butadiene is a very characteristic emission from both cyclic alkane and terminal straight chain olefin fuels as shown in Table II and expanded upon in reference[10] (e.g. butadiene from

1-hexene fuel is 10% of the total emissions at baseline). Little butadiene (0.7% at baseline) is formed from a branched olefin such as diisobutylene or the paraffins n-butane (0.8%) or isooctane (0.5%). Thus, these experiments have clearly identified potential sources of butadiene emission from gasoline fuel.

Table III. Exhaust mole fraction (ppmC_1) of toluene for three engine operating conditions using pure toluene and a 53%/47% toluene-hexane mixture.

operating condition	toluene fuel	hexane/ toluene	predicted for mix[a]
base	1763	669	936
retarded spark	1215	408	645
fuel rich	2807	1292	1491

[a] Predicted toluene mole fraction using the measured exhaust toluene for toluene fuel scaled by the known (53.1%) amount of toluene in the mix.

ADDITIVITY OF FUEL COMPONENTS IN A MIXTURE - To predict HC emissions of fuel mixtures based on measured emissions from pure fuels, additivity of fuel components must be established. Ideally, if an engine is run on a 50/50 mixture of fuel components, the exhaust HC species emissions from each component would be one half of those from the pure fuel. In some cases, this is observed. As an example, when 20% diisobutylene (DIB) was added to gasoline, the emissions arising from DIB could be predicted well from the measured emissions from pure DIB and the known amount of DIB in the fuel mixture.[10] However, additivity is not always observed. Table III presents the mole fraction of toluene in the exhaust for two fuels: pure toluene; and a blend of toluene with n-hexane. Based on the measured mole fraction of toluene from the pure fuel at three engine operating conditions and the known amount of toluene in the mixture, predictions of the expected exhaust toluene emission can be made. It is evident that in this case, the prediction is not as accurate. In all cases, the exhaust toluene is predicted to be substantially larger than is observed. Therefore, addition of a more volatile fuel to toluene actually reduces the emissions from the toluene in the mixture. In another experiment, a very low volatility component (1,2,4-trimethyl benzene) was added to gasoline. In this case, the emissions of all components in the gasoline, including high volatility ones (e.g. isopentane), increased relative to expectation,[17] opposite to the result in Table III. Thus, it appears that if the vaporization characteristics of a fuel are changed by addition of another fuel component, the emissions may not follow a simple additivity relationship. Increasing the volatility decreases the emissions even of low volatility components. Decreasing the volatility increases the emissions even of high volatility components. In the DIB example, DIB has a volatility approximately equal to the mid-point of gasoline. Thus, the volatility of the mixture was not changed significantly from that of the base gasoline, and this may explain why additivity was observed. Similar conclusions were drawn during the AUTO/OIL Air Quality Improvement Research Program[18] in which it was observed that "the presence of heavy hydrocarbons in the fuel seems to increase the mass emissions of all unreacted fuel species of a given hydrocarbon class equally, regardless of boiling point." These Auto/Oil tests were performed on a wide variety of engine designs.

SUMMARY

In this presentation, the sources of hydrocarbon emissions have been discussed, showing the importance of crevice storage, in-cylinder wall wetting by fuel, and burn-up of stored fuel late in the engine cycle in determining both total emissions and the distribution of individual species. Engine operating conditions influence exhaust HC emissions. Conditions that result in higher in-cylinder or exhaust temperatures (e.g. higher speed or retarded spark timing) result in reduced total HC emissions accompanied by an increase in the importance of partial oxidation products such as olefins. The volatility of the fuel can affect emissions of all components in a gasoline. These observations indicate that the emissions process is a complicated one that will be difficult to model with accuracy for any given vehicle. However, the experiments summarized above show that the sources of particular exhaust gas species can be evaluated effectively by pure fuel experiments. Thus, benzene is formed from substituted aromatic fuel components and from cyclic alkanes. Terminal, straight-chain olefins and cyclic alkanes form substantial amounts of butadiene. While cyclic alkanes are not normally present in large quantity in gasoline and the concentrations of olefins are being reduced, particularly in California, it is important to understand the emissions properties of all types of gasoline fuel components to avoid changing gasoline formulation in ways that will be detrimental to the environment. It is also critical to understand the effect of engine calibration (speed, spark timing, fuel/air ratio, etc) on the hydrocarbon emissions process in order to most efficiently meet government regulations, whether they are based on total HC mass or on atmospheric reactivity.

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TOXICITY EVALUATION OF GASOLINE EXHAUST EMISSIONS.

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INTRODUCTION

The health effects of gasoline exhaust was examined previously in two animal toxicology studies (Brightwell et al., 1986a, 1986b, 1989; Heinrich et al., 1986). These studies were part of larger efforts examining the health effect of gasoline or diesel exhausts. The Brightwell study examined the systemic and pulmonary toxicity and carcinogenicity in rats and hamsters of engine-out (uncatalyzed) and tailpipe (catalyzed) exhaust emissions from engines burning unleaded gasoline. Two studies by Heinrich examined the subchronic and chronic toxicity and carcinogenicity in rats and hamsters of engine-out exhaust emission using leaded gasoline.

The results of Brightwell and Heinrich studies provide no evidence that gasoline engine exhaust is an animal carcinogen. The strongest dose-response and time-response relationships observed in these studies were linked to carbon monoxide (CO) exposure. The authors of these reports speculated that the respiratory and histopathological changes of the lung were associated with nitrogen oxides (NOx), CO or lead particulate. Although some uncertainty exists in this analysis, the lesions that were observed are typical of these toxicants. It is well known that CO has marked effects on hematological and cardiac indices (USEPA, 1991). Nitrogen oxides are known to adversely affect the respiratory tract (USEPA, 1993). Thus, although Brightwell and Heinrich speculated on these findings, this hypothesis appears reasonable based on the known toxicity of CO and NOx.

Automotive gasoline composition has changed since the time these studies were conducted. Oxygenated winter fuel (oxyfuel) and reformulated gasolines (RFG) were introduced in areas of CO or CO and ozone nonattainment, respectively, in the 1990s. These fuels differ from regular gasoline by the addition of an oxygenate to promote a more efficient combustion of fuel. Oxygen content of RFG and oxyfuels are be 2.0 and 2.7 wt. percent, respectively resulting in 8 to 17 volume percent of gasoline depending on the oxygenate. With the introduction of these fuels into the marketplace, there has been concern regarding the toxicity of the combustion emissions of these fuels.

Components of automotive engine exhaust have been characterized. The Auto/Oil Air Quality Improvement Research Program (AQIRP) developed an extensive database on the level and composition of exhaust and evaporative emissions from up to 20 well-maintained model year 1989 cars and light trucks operated on industry average gasoline (RFA), and gasoline blended with the oxygenates methyl-tertiary-butyl ether (MTBE), ethanol (EtOH) and ethyl-tertiary butyl ether (ETBE). From the AQIRP speciation data, extrapolations can be made to determine if the toxicity of gasoline exhaust emissions has changed, and if further animal toxicology testing will to characterize gasoline exhaust toxicology will provide additional information from that which can be inferred from the above studies.

METHODS

Two sets of data from AQIRP were used for this paper. AQIRP Pilot Study includes both engine-out and tail-pipe exhaust measurements of 156 hydrocarbon and oxygenated species sampled and composited over three phases of the FTP driving cycle. The Phase I Working Data Set includes exhaust tailpipe measurements of 156 hydrocarbon and oxygenated species, sampled and composited over three phases of the FTP driving cycle. Detailed descriptions of the data in these two data sets are presented in technical papers contained in SAE Publication SP-920, *Auto/Oil Air Quality Improvement Research Program,* February 1992.

AQIRP data (g/mile) can be used to estimate concentrations in an inhalation toxicology study (ppm) by developing a molar ratio between a particular hydrocarbon species and CO. As CO will be the limiting exhaust component, hydrocarbon/CO molar ratio can be

multiplied by the fixed concentration of CO to produce the estimated exposure concentration of hydrocarbon. The conversion is illustrated in the equation below.

$$\left(\frac{\left([HC_{exh}] \div HC\,mol.wt.\right)}{\left([CO_{exh}] \div CO\,mol.wt.\right)}\right) \times \left[CO_{exp\,chamb}\right] = \left[HC_{exp\,chamb}\right]$$

Where $[HC_{exh}]$ is the measured concentration in mg/mile of a hydrocarbon species from Auto/Oil; HC mol. wt. is the molecular weight for a given hydrocarbon species; $[CO_{exh}]$ is the measured concentration in mg/mile of carbon monoxide from Auto/Oil; CO mol. wt. is the molecular weight of carbon monoxide; $[CO_{exp\ chamb}]$ is the expected chamber concentration of carbon monoxide set at 200 ppm; and $[HC_{exp\ chamb}]$ is the expected animal exposure chamber concentration of a particular hydrocarbon species in ppm.

The entire speciated exhaust component data sets were converted. As noted above the expected chamber concentration of CO was set at 200 ppm based on a large number of toxicity endpoints (developmental and systemic) following review of the health effects of CO. The expected exposure concentrations from the converted AQIRP data were compared to exposure concentrations from previous studies.

Toxicology databases were searched to determine No Observable Effect Levels (NOELs) for exhaust components derived from animal toxicity studies. NOELs represent exposure concentrations at which no effects were observed in an animal toxicity study. NOELs were compared to expected exposure concentrations from the converted AQIRP data. Only a few representative exhaust components are presented due to space limitations.

RESULTS

Based on a dose limiting concentration of 200 ppm for CO, the expected chamber concentrations of CO, CO_2, NOx and total hydrocarbon can be extrapolated from AQIRP data using the equation listed above (Table 1). These data demonstrate that for a CO concentration of 200 ppm, total hydrocarbon concentrations will be approximately 70 ppm for RFA gasoline. These data are similar to data obtained in the Brightwell and Heinrich studies, which also are listed in this table.

Hydrocarbon speciation data from the Brightwell study can be directly compared to extrapolated data from AQIRP (Table 2). The extrapolated AQIRP data have been converted from ppm to mg/m^3 to make a direct comparison to Brightwell data easier. The extrapolated data is not directly comparable as the Brightwell data is the average and range of ten measurements on one vehicle over the course of two years, and AQIRP data is the average and range of two FTP tests for three vehicles. However, it is apparent that the expected concentrations for individual hydrocarbons is similar to what was actually measured in the Brightwell study.

Comparison of extrapolated animal exposure concentrations of hydrocarbon species for RFA and oxygenated fuel mixtures MTBE, EtOH or ETBE gasoline (Table 3) indicates that the addition of oxygenate to gasoline produces minor alterations in the composition of gasoline exhaust. As seen with the engine-out data, parent oxygenate was observed in the exhaust stream, at concentrations of approximately 1 ppm. Additionally, the concentration of aldehydes was increased with the addition of oxygenate, most notably formaldehyde and acetaldehyde. The remaining hydrocarbon species are not affected greatly by addition of oxygenate.

Extrapolated animal exposure concentrations of speciated exhaust components are compared to these components NOELs in Table 4. The comparison indicates that the exposure concentrations will be well below the observable effects level for all components.

DISCUSSION

Extrapolation of the AQIRP data indicate that total hydrocarbon concentrations will be low in animal exposures using RFA gasoline. These data also indicate the hydrocarbon exposure levels will be similar to the levels observed in the previous gasoline engine exhaust toxicology studies, where only CO and/or NOx effects were observed.

Small analytical differences in speciated exhaust components do exist between the extrapolated AQIRP data for gasoline and gasoline with MTBE and the speciated data measured in the Brightwell study. However, the composition of the exhaust atmosphere between the two data sets are fairly similar. This is not entirely surprising since the specifications for the fuel used in the Brightwell study are similar to the specifications for RFA used in AQIRP. Thus, results observed in the Brightwell study appear to be applicable to exhaust emissions generated from RFA and gasoline with MTBE. Further, although there are slight analytical differences the anticipated animal exposure atmospheres for the different reformulated fuels, exposures to exhaust components in toxicology studies on reformulated or oxygenated fuels will be fairly similar to the exposures in the Brightwell study. Thus, it can be concluded that addition of oxygenate does not dramatically alter the subsequent composition of an animal exposure to gasoline engine exhaust. Therefore, the Brightwell study results can be used to evaluate the health effects of engine exhaust from oxygenated gasolines.

Comparison of anticipated animal exposure levels and NOELs for exhaust components indicates no adverse effects are likely to be observed. Dilution of engine exhaust to reduce CO toxicity provides an inherent safety facto for the hydrocarbon component of exhaust. At 200 ppm CO it is unlikely that hydrocarbon effects will be observed as the anticipated exposure levels are well below the NOELs for these compounds. Doubling the CO concentration to 400 ppm will not sufficiently increase the hydrocarbon concentration above the NOEL fot the individual hydrocarbons.

CONCLUSION

Although slight analytical differences exist in exhaust HC compositions for different oxygenated fuel blends, the likelihood of discerning differences in the toxicity of HC exhaust emissions from different gasoline blends is small.

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Table 1 Extrapolated and Measured Combustion Gas Concentrations (ppm)			
Compound	AQIRP	Brightwell	Heinrich
CO	200	224	305
Total Hydrocarbon	68	61	36.5[1]
NO_x	32	49	23[2]
CO_2	4200	6000	4000

[1] Non-methane hydrocarbon
[2] Measured as NO

Table 2 Concentration of Selected Hydrocarbons From Measured and Extrapolated Data			
	Brightwell	AQIRP Pilot Study	
Fuel Type	California	Baseline Gasoline	Gasoline with MTBE
Units	mg/m^3	mg/m^3	mg/m^3
Compound	Range (Average)	Range (Average)	Range (Average)
Methane	1.90 - 4.94 (2.96)	1.00 - 1.08 (1.03)	1.02 - 1.14 (1.09)
Benzene	0.45 - 2.26 (1.39)	1.27 - 1.53 (1.39)	1.13 - 1.26 (1.20)
Toluene	2.59 - 6.86 (4.1)	2.00 - 2.10 (0.82)	1.36 - 1.51 (1.45)
Formaldehyde	0.104 - 0.590 (0.308)	0.48 - 1.15 (0.82)	0.71 - 1.03 (0.91)
Acetaldehyde	0.073 - 0.297 (0.148)	0.20 - 0.35 (0.26)	0.20 - 0.29 (0.24)
MTBE			1.65 - 2.16 (1.95)

Table 3 Expected Hydrocarbon Chamber Concentration (ppm) for Baseline Gasoline, Gasoline/MTBE, Gasoline/EtOH, Gasoline/ETBE at 200 ppm CO				
Hydrocarbon	Gasoline	Gasoline/MTBE	Gasoline/EtOH	Gasoline/ETBE
Methane	1.57	1.72	1.71	1.71
Toluene	0.71	0.75	0.68	0.72
Formaldehyde	0.55	0.49	0.41	1.35
Benzene	0.33	0.34	0.32	0.44
13-Butadiene	0.17	0.20	0.18	0.20
Acetaldehyde	0.14	0.15	0.29	0.43
E-Benzene	0.13	0.13	0.12	0.15
o-Xylene	0.09	0.09	0.08	0.10
ETBE	0.02	0.02	0.02	1.22
MTBE	0.00	1.24	0.00	0.00
EtOH	0.00	0.00	1.35	0.00

Table 4
Comparison of NOELs and Expected Chamber Concentration for Selected Hydrocarbons

Compound	Chamber Conc. (ppm)	NOEL[1] (ppm)	Endpoint	Species	NOEL to Exposure Ratio
Formaldehyde	0.580	15 mg/kg[2] 74 mg/kg[2]	Systemic Developmental	rat rat	
Acetaldehyde	0.145	150	Systemic	rat	1000
1,3-Butadiene	0.351	6.25[3]	Reproductive	mouse	18
Benzene	0.436	10 300 30 300	Developmental Reproductive Reproductive Neurological	rat rat mouse mouse	23 700 70 700
Toluene	0.545	56 500 750	Neurological Reproductive Developmental	rat rat rat	100 1000 1400
m- and *p*-Xylene	0.407	99 250 1000	Neurological Developmental Reproductive	rat rat rat	240 600 2500
Ethylbenzene	0.188	100 100	Developmental Systemic	rat rat	500 500
MTBE	0.542	1000 1000 400	Reproductive Developmental Neurological	rat mouse rat	1900 1900 200
Ethanol	1.35	20,000	Developmental	rat	15,000
ETBE	1.22	500	Neurological	rat	400

[1] No Observable Effect Level
[2] Oral Exposure
[3] Low Observable Effect Level

PREDICTING THE TOXICITY OF GASOLINE VAPORS BASED ON KNOWLEDGE OF FUEL COMPONENTS

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ABSTRACT

The toxicity of chemicals in mixtures such as gasoline may differ greatly from that observed when the chemicals are tested as pure compounds. For example, metabolic activation is the critical first step in the development of toxicity after exposure to benzene. Gasoline components inhibit benzene metabolism and thus reduce benzene's toxicity. The extent to which inhibition occurs depends on the gasoline vapor composition and inhaled concentration. Gasoline vapors vary in inhibitory effects based on the aromatic content of the mixture. Additionally, metabolic inhibition is dependent on concentration, with inhibition increasing with increasing concentration. The challenge in estimating the effect of gasoline components on the inhibition of benzene metabolism is to determine the shape of the concentration-inhibition function.

INTRODUCTION

Benzene is a ubiquitous industrial and environmental pollutant (Runion and Scott, 1985). It is present in automobile emissions, both evaporative and combustive, and has been detected in cigarette smoke (Wallace, 1990; Wallace and Pellizzari, 1986). Exposure to benzene is most likely associated with coexposure to other volatile organic chemicals normally present in the environment.

Benzene is myelotoxic and carcinogenic at high concentrations. Epidemiology studies have shown that people develop blood dyscrasias, such as pancytopenia, aplastic anemia, and acute myelogenous leukemia following repeated exposure to high concentrations of benzene (Goldstein, 1977; Rinsky et al., 1987). Cytogenetic damage has been observed in humans who have developed benzene-associated hemopathies, especially leukemia (Huff et al., 1989). This correlation between cytogenetic damage in leukemia suggests that cytogenetic alterations in bone marrow cells may be a good marker for genetic alterations in bone marrow stem cells that precede the development of leukemia.

Benzene is not thought to be a direct-acting agent in the bone marrow, but rather is converted to bioactive metabolites (in the liver) which cause myelotoxicity (Irons, 1985; Eastman et al., 1987; Barale et al., 1990). The metabolism of benzene involves a series of oxidations of the benzene ring by cytochrome P450 monooxygenases (Figure 1). After absorption into the blood and translocation to the liver, benzene is metabolized by cytochrome P450 2E1 to its major metabolite, phenol (Figure 1; Smith et al., 1989). Phenol is be further oxidized by the same cytochrome P450 to the polyhydroxylated metabolite, hydroquinone (Koop et al., 1989; Schlosser et al., 1993). Both phenol and hydroquinone can translocate in the blood to the bone marrow where they interact with critical blood cell components. Alternatively phenol and hydroquinone are detoxified by Phase II conjugating enzymes such as sulfotransferases and glucuronyl transferases. Muconaldehyde, a reactive metabolite of benzene, is also thought to be formed by a two step oxidation of benzene although the mechanism and isozyme involved are unknown.

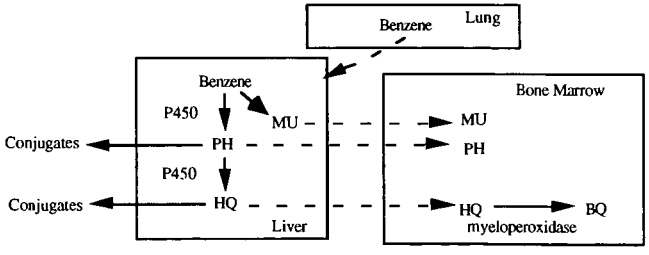

Figure 1. Metabolic scheme for benzene and its major metabolites. BQ = Benzoquinone, HQ = Hydroquinone, PH = Phenol, MU = Muconaldehyde

INTERACTIONS WITH OTHER VOLATILE ORGANICS

The multiplicity of benzene's metabolic pathways provides opportunities for modulation of benzene metabolism, either by competition with other organic chemicals for available

enzyme sites, by induction or inhibition of the oxidation or conjugation enzymes, or by direct competition among benzene and its metabolites for enzyme sites. Other volatile organics can modulate the toxicity and metabolism of benzene. Gad-El-Karim et al. (1984) investigated the genotoxicity of benzene in mice treated orally with benzene or combinations of benzene and toluene. Benzene alone was clastogenic to bone marrow cells and elevated numbers of micronucleated polychromatic erythrocytes (micronuclei, MN) were detected in mice receiving benzene compared with controls. When both benzene and the aromatic hydrocarbon, toluene, were coadministered, the clastogenic effect of benzene was reduced considerably. Similar results were noted when chromosomal aberrations were analyzed. These investigators hypothesized that toluene inhibited the metabolism of benzene and that one or more metabolites of benzene were responsible for the myeloclastogenic effects.

Andrews et al. (1977) used incorporation of ^{59}Fe into maturing red blood cells to evaluate the effects of benzene on erythropoiesis. Mice were given injections of benzene alone or combinations of benzene plus toluene. In parallel studies, the effect of toluene on the pharmacokinetics of benzene and its metabolites was also investigated. Coadministration of toluene and benzene resulted in reduction in the quantity of benzene metabolites measured in urine compared with the benzene-only exposed group. Unmetabolized benzene was exhaled. Coexposure to toluene also counteracted the benzene-induced reduction in ^{59}Fe uptake. Thus, toluene both reduced benzene metabolism and protected against benzene-induced suppression of iron utilization by red cells. The concentration of benzene in bone marrow was similar in mice given only benzene compared with mice given benzene and toluene. In contrast, concentrations of benzene metabolites in bone marrow of mice given benzene alone were much higher than those found when benzene was coadministered with toluene. In summary, the observation that toluene reduced both the level of benzene metabolites and the inhibition of iron uptake suggests that metabolism of benzene is closely related to its hematotoxicity. Toluene protects against benzene-induced hematotoxicity by reducing the level of benzene metabolites in bone marrow through suppression of benzene metabolism.

Mutual metabolic suppression between benzene and toluene also occurs in people. For example, Inoue et al. (1988) examined both the exposure concentration during a workshift and the benzene metabolite concentrations in urine of male Chinese workers exposed to either benzene, toluene, or a mixture of both chemicals. Urinary levels of the benzene metabolites phenol and hydroquinone were lower in the workers exposed to both toluene and benzene compared with those exposed to benzene alone. The investigators hypothesized that biotransformation of benzene to its hydroxylated metabolites in people is suppressed by coexposure to toluene.

Gasoline, one important source of environmental exposure to benzene, also contains toluene and other aromatic and aliphatic hydrocarbons such as xylene and hexane. These hydrocarbons could inhibit benzene metabolism. Travis et al. (1992) examined the effect of coexposure to gasoline vapor on the metabolism of benzene. Coexposure to gasoline vapors increased the maximum rate for benzene metabolism and decreased the apparent affinity of the enzyme for benzene. Generally, enzymatic inhibition is associated with a decrease in the maximum rate and an increase in the apparent affinity. Thus, the results of this study are in contrast with the demonstration of the inhibition of benzene metabolism by toluene previously reported by these investigators using a similar experimental system (Purcell et al., 1990). Clearly more research on the interaction of benzene with gasoline components is necessary to adequately assess the potential human health risks for this environmentally important mixture.

MODEL SIMULATIONS OF BENZENE-GASOLINE INTERACTIONS

Figures 2 and 3 show the results of physiologically-based pharmacokinetic model simulations of four different benzene-gasoline exposure scenarios. Figure 2 assumes that the major gasoline components are aromatic chemicals. Figure 3 assumes that the major gasoline components are aliphatic chemicals. Simulations in the A panels are for an exposure to gasoline at the threshold limit value (300 ppm) and simulations in the B panels are for an exposure at 2000 ppm, the concentration used in the chronic toxicity studies with unleaded gasoline. Comparison of simulations for the aromatic and aliphatic volatiles at the high doses (Panel B) compared with the lower dose (Panel A) demonstrates the dose dependence of inhibition by gasoline components on benzene metabolism. Less benzene is metabolized (more inhibition) at the higher (B) compared with the lower (A) dose. This can be determined by comparing the differences for the simulations for benzene alone to benzene plus other volatiles for each figure. At the low dose, the difference is small; at the high dose, the difference is large.

A comparison of the relative effect of gasoline vapor composed primarily of aromatics to that composed primarily of aliphatics can be seen by comparing results of model simulations at the low dose (Figures 2A and 3A). The aromatic chemicals appear to be better inhibitors of benzene at this lower concentration than do aliphatics. Again, this can be seen by the difference in the predicted benzene metabolized when benzene is given alone compared with the predictions when benzene plus other volatiles are given. The results of these simulations suggest that the scientific answer to the question, "Does gasoline inhibit benzene metabolism?", is that inhibition depends both on dose and on chemical composition.

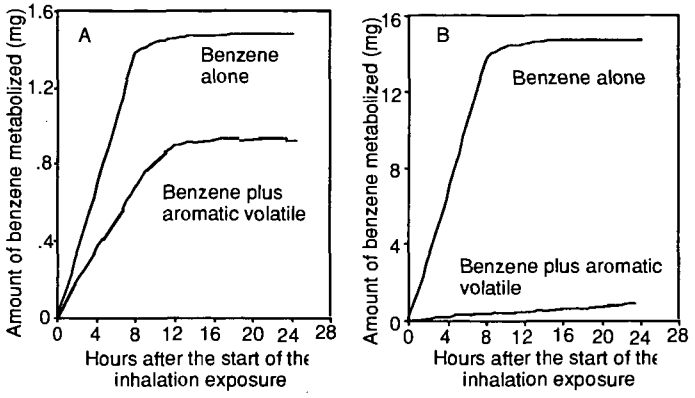

Figure 2. Model simulations for the effect of aromatic volatiles on the metabolism of benzene by one mouse after an 8-hr exposure to (A) 300 ppm of aromatics together with 5 ppm benzene or to a 5 ppm benzene exposure only or to (B) 2000 ppm of aromatics together with 40 ppm benzene or to a 40 ppm benzene exposure only.

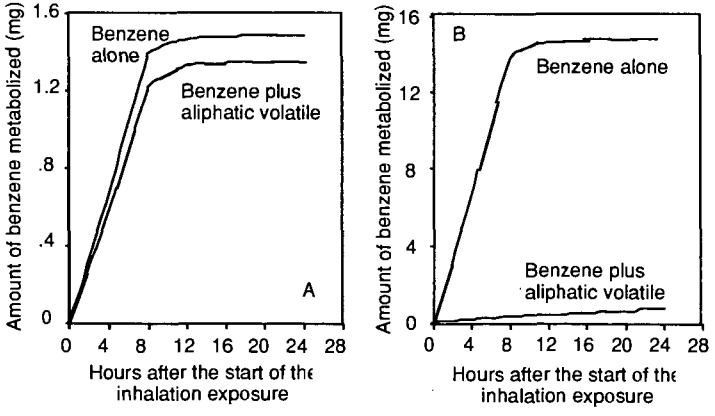

Figure 3. Model simulations for the effect of aliphatic volatiles on the metabolism of benzene by one mouse after an 8-hr exposure to (A) 300 ppm of aliphatics together with 5 ppm benzene or to a 5 ppm benzene exposure only.or to (B) 2000 ppm of aliphatics together with 40 ppm benzene or to a 40 ppm benzene exposure only.

CONCLUSIONS

There are two major factors that influence the potential interaction of benzene and gasoline components. These issues relate to the composition of the gasoline vapor and the inhaled concentration (or dose) of the vapor. Some components of gasoline may be better inhibitors of benzene metabolism than are others. The aromatic components, such as xylene and toluene, may be more effective competitors against benzene for active enzyme sites by nature of their greater solubility in tissues compared with benzene. Concentrations of these chemicals might be higher in the metabolizing organs such as liver and therefore these aromatics might be better able to compete with the benzene for active metabolic sites.

The fractional composition of volatile gasoline components changes with increasing vaporization temperature. Of most importance is the composition of the vaporized fuel relative to the whole gasoline. With increasing temperature, the aliphatic volatile organics make up an increasing percentage of the total percent of the gasoline organics and the aromatics become a decreasing percent. The scientific question relevant for risk assessment relates to the range of composition of vapors that people are likely to be exposed to when using gasoline.

Inhibition of one chemical on the metabolism of another is typically a dose-dependent phenomenon. At very high doses, inhibition can approach 100%. At very low doses, inhibition may be insignificant. The challenge in estimating the effect of gasoline components on the inhibition of benzene metabolism is to determine the shape of the concentration-inhibition function. The added complexity is that the shape of the benzene concentration-target tissue dosimetry function is also highly nonlinear. In actuality, it is not just the effect of gasoline components on the total benzene metabolized that must be addressed, but also the effect of possible inhibition of the formation of oxidized metabolic products such as hydroquinone.

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HEALTH RESEARCH DIRECTIONS FOR NEW FUELS.

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INTRODUCTION

Throughout much of the twentieth century, the general population has been routinely exposed to conventional gasoline and its evaporative and combustion emissions. However, with the introduction of new fuel additives and formulations, attention has become focused in recent years on the potential for public health impacts from widespread exposure to motor vehicle fuels. The Clean Air Act required the Administrator of the U.S. Environmental Protection Agency (EPA) to promulgate requirements for testing the health effects of evaporative and combustion emissions of fuels and fuel additives (F/FAs). The Fuels and Fuel Additives Rule, promulgated on May 27, 1994, established new health testing requirements for the registration of designated F/FAs, organized within a three-tier structure. Tier 1 requires F/FA manufacturers to perform a literature search on the health and welfare effects of F/FA emissions and to characterize F/FA emissions. Tier 2 requires toxicological testing by subchronic inhalation exposure and designated assays for specific health endpoints if adequate information is not already available. When necessary, Tier 3, which may include follow-up or additional studies, can be required.

Additionally, the rule includes a provision known as Alternative Tier 2, which gives EPA the flexibility to prescribe additional tests to be performed along with the standard Tier 2 program, to substitute different tests, and/or to modify the underlying vehicle/engine specifications for Tier 2. EPA may also use the Alternative Tier 2 authority to waive certain Tier 2 endpoint evaluations (generally on occasions when additional and/or more rigorous tests are being required for other Tier 2 endpoints). However, testing for Tier 2 endpoints may not be waived in the absence of adequate information or requirements for more rigorous testing.

At the time of this writing, EPA is about to issue proposed Alternative Tier 2 testing requirements for baseline (conventional) gasoline and various oxygenate-gasoline blends (collectively referred to here as "oxyfuels"), including methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), ethyl alcohol (EtOH), tertiary amyl methyl ether (TAME), diisopropyl ether (DIPE), and tertiary butyl alcohol (TBA). The primary objective of this testing program is to develop an information base that will support quantitative, comparative risk assessments of baseline and oxygenated gasolines. The risks, and benefits, of a given fuel are relative to its alternatives. Therefore, to determine whether a particular oxyfuel is better or worse than conventional gasoline or some other oxyfuel, comparable data must be available by which to evaluate the comparative risks. More extensive discussions of needed information and research relative to oxyfuels may be found in EPA's "Oxyfuels Information Needs" (U.S. Environmental Protection Agency, 1996) and other recent reports (e.g., Health Effects Institute, 1996; Interagency Oxygenated Fuels Assessment Steering Committee, 1996, 1997; National Research Council, 1996). This paper will explain the rationale underlying the proposed Alternative Tier 2 testing program and summarize key features of the program as an illustration of a scientifically sound and efficient approach for obtaining data needed for comparative risk assessment purposes.

RATIONALE FOR ALTERNATIVE TIER 2 TESTING REQUIREMENTS

To understand the rationale behind the Alternative Tier 2 testing program, one must first understand the purpose of standard Tier 2 requirements in the F/FA rule. Standard Tier 2 assessments include a basic subchronic inhalation toxicology study as well as tests to determine potential reproductive, developmental, neurotoxic, mutagenic, and carcinogenic effects (summarized in Table I). These assays, while sufficient for screening level evaluations of the toxicological effects of inhalation exposure to the emissions of designated F/FAs in test animals, were not intended necessarily to provide an adequate base for quantitative risk assessments. Rather, the intent was to provide for the collection of basic toxicological data that, along with information on exposure potential and other considerations, could guide decisions on whether or not more extensive toxicological evaluation would be required. If the results from standard Tier 2 assays indicated low toxicity for a particular F/FA and little potential for human exposure existed, then further testing would probably not be warranted. However, in the case of oxyfuels, a testing regimen that exceeds the standard screening requirements of Tier 2 is considered necessary and appropriate because of continuing uncertainties regarding the public health effects of gasoline and oxyfuels, and the widespread public exposure to these fuels and related emissions. This approach is clearly more cost-effective and time-efficient than simply requiring standard Tier 2 testing, waiting for the completion of such testing, and then

Table I. Fuel/Fuel Additive (F/FA) Rule Standard Tier 2 Tests

90-Day Subchronic Inhalation General Toxicity: Screening information on target organ toxicities and on concentrations useful for running chronic studies.

30 rodents per concentration per group (add specified numbers for different assessments combined with general toxicity); recovery group (N = 20) observed for reversible, persistent, or delayed effects

Observation (including body weight)
Clinical exams: hematology (e.g. Hct, Hb, RBC, DLC); clinical biochemistry (e.g., electrolyte balance, liver and kidney function, Ca-P-Cl-Na-K, glucose, BUN)
Ophthalmological exam
Urinalysis
Gross pathology
Histopathology (especially respiratory tract)

Fertility/Teratology: Information on potential health hazards to fetus and on gonadal function, conception, and fertility.

25 males, 40 females per group; mating after 9 weeks of exposure, then exposure of females continues through GD 15
Limit test (if no effects at highest concentration, then omit lower concentrations)

Observation for ≤13 weeks
Vaginal cytology
Mating and fertility
Gross necropsy (especially including reproductive organs)
Fetal anomalies, resorptions
Histopathology of reproductive organs

In Vivo Micronucleus: Detect damage to chromosomes or mitotic apparatus of cells (based on increase in frequency of micronucleated RBCs); provides information on potential carcinogenic and/or mutagenic effects.

5 females and 5 males per group
Positive control

In Vivo Sister Chromatid Exchange: Detect enhancement of exchange of DNA between two sister chromatids of a duplicating chromosome (using peripheral blood lymphocytes grown to confluence in cell culture); provides information on potential mutagenic and/or carcinogenic effects.

5 females and 5 males per group
Positive control

Neuropathology: Provides data on morphologic changes in central and peripheral nervous system.

N = 10 per group; N = 20 for reversible, persistent, or delayed effects
Positive control
Limit test (if no effects at highest concentration, then omit lower concentrations)

Observation (including body weight, movement disorders, etc.)
Brain size and weight; light (and possible electron) microscopy of sections
Peripheral nerve teasing

Glial Fibrillary Acidic Protein: An indicator of neurotoxicity associated with astrocytic hypertrophy at site of damage.

10 animals per group
Change in amount of GFAP for specific brain region as a function of treatment and dose

***Salmonella Typhimurium* Reverse Mutation:** Microbial assay that measures histidine (*his*) reversions (*his*$^-$ to *his*$^+$), which cause base changes or frame-shift mutations in the genome; provides data on mutagenicity.

Positive controls
Data presented as number of revertant colonies per plate, per kilogram (or liter) of fuel, and per kilometer (or mile) for each replicate and dose.

Source: U.S. Environmental Protection Agency (1996)

developing follow-up test requirements at the Tier 3 level that would be necessary to support quantitative, comparative risk assessments.

Although both evaporative and combustion emissions are encompassed by the F/FA rule, the proposed test program focuses on evaporative emissions of the fuels. A full discussion of the reasons for deferring combustion emissions testing is outside the scope of this paper, but the evaporative emissions amply serve to illustrate a testing strategy for new F/FAs. The proposed Alternative Tier 2 test program has been structured so as to obtain rather extensive data on baseline gasoline. Several considerations have led EPA to propose more extensive test requirements for baseline gasoline and MTBE-gasoline than for the other oxygenates. First, and most important, conventional gasoline and MTBE-gasoline predominate within the U.S. fuel marketplace, and thus present the highest potential for human and environmental exposures. A thorough understanding of the individual and comparative public health risks of these fuels thus constitutes a critical need. Second, the fact that nearly all fuels have some degree of toxicity means that the relative risk of different fuels is particularly important. Accordingly, a comprehensive database on baseline gasoline toxicity is vitally needed to provide a level basis for comparison with other F/FAs in the gasoline family. Similarly, since MTBE is the most frequently used oxyfuel, comprehensive data on MTBE-gasoline is needed not only in comparison with baseline gasoline but also to provide an additional reference point for evaluating the relative toxicity of other oxyfuels.

Third, previous scientific work on conventional gasoline and on MTBE has identified specific information gaps which cannot be satisfactorily addressed by the short-term screening tests required under Standard Tier 2. For example, the comparative carcinogenic potential of baseline gasoline emissions relative to those of MTBE-gasoline emissions is an outstanding fundamental issue which must be evaluated in the context of long-term emission exposures. In addition, dose-response relationships for developmental, reproductive, and neurotoxic effects have not been adequately characterized. Fourth, even though each oxygenate has its own chemical characteristics and, perhaps, toxicological potencies, the test results obtained on one such fuel can still help to inform the Agency's decision-making about potential testing needed on other oxyfuels. For example, if certain test results for baseline gasoline and MTBE-gasoline are negative, this may support the validity of negative results for analogous screening tests on other oxyfuels. On the other hand, a positive result obtained on MTBE-oxyfuel under relatively rigorous study conditions may indicate that comparative results are needed for the other oxyfuels. These illustrations help explain why the more extensive set of requirements are initially to be applied on a selective basis to baseline gasoline and MTBE-gasoline, rather than applying the same, relatively stringent set of Alternative Tier 2 requirements across the board to all registered oxyfuels.

SUMMARY OF ALTERNATIVE TIER 2 TESTING REQUIREMENTS

For baseline gasoline and MTBE-gasoline, the standard Tier 2 testing regimen is to be supplemented by (1) two additional neurotoxicity assessments, the functional observational battery and motor activity assessment; (2) a two-generation reproductive study; (3) a two-species developmental study; (4) a two-year carcinogenicity study; and (5) a screening panel for immunological effects. (The two-generation reproductive study and two-species developmental study replace the Standard Tier 2 fertility/teratology combined screening assessment.) The testing requirements for the other oxyfuels are much less extensive, consisting of the Standard Tier 2 requirements modestly expanded to include a screening panel for immunological effects and certain histopathological requirements. Because there is a paucity of inhalation toxicity data on these other oxyfuels, the screening level studies required in Standard Tier 2 are appropriate for determining whether additional studies are necessary. The results of these studies will determine whether additional studies are required at the Tier 3 level.

The Alternative Tier 2 program also incorporates provisions for pharmacokinetic studies on "neat" oxygenates. An understanding of the pharmacokinetic characteristics of the oxygenates as pure compounds is important to our understanding of their relative toxicities when mixed in gasoline. Basic pharmacokinetic characterization (i.e., absorption, distribution, metabolism, and elimination of the pure inhaled oxygenates) can provide mechanistic information on disposition that will be useful to determining whether the toxicological testing results for one oxyfuel (e.g., MTBE-gasoline) can be compared with another. Such studies can also shed light on the relevance of animal-based study results to humans and help determine the extent to which effects by one route of exposure may be relevant to another route.

Comprehensive inhalation pharmacokinetic studies have already been conducted for MTBE; therefore, additional such testing is not required. But, the availability of inhalation pharmacokinetic data for the other oxygenates varies considerably. For example, pharmacokinetic studies are already underway for TAME. In addition, EPA has been informed that such testing on pure ETBE is being conducted by industry on a voluntary basis. To our knowledge, however, there are currently no

similar test plans for pure EtOH, DIPE, TBA, or other oxygenates. Consequently, the proposed Alternative Tier 2 test regimen for the oxygenates other than MTBE includes pure compound inhalation pharmacokinetic test requirements.

CONTINGENT STUDIES

As discussed above, the proposed Alternative Tier 2 testing program has been designed to fill critical data gaps and act as a screen to determine the need for additional studies. Thus, the results of the Alternative Tier 2 tests may indicate that additional studies are required at the Tier 3 level. In the case of baseline gasoline and MTBE-gasoline, follow-up tests may be required to further characterize significant unexpected positive findings. For example, mechanistic studies may be required to determine if positive results of concern in the Alternative Tier 2 animal studies were applicable to humans.

In the case of the other oxyfuels, additional testing may be required for a particular gasoline-oxygenate mixture, not only to explicate Alternative Tier 2 positive results on the mixture in question, but also to resolve uncertainties created by positive results that might be obtained on MTBE-gasoline, another oxygenate mixture, and/or baseline gasoline. Similarly, a two-year inhalation bioassay may be required if either positive results are obtained in the Alternative Tier 2 mutagenicity studies for a given oxyfuel or if significant unexpected results are obtained in the cancer bioassay conducted for baseline gasoline and/or MTBE-gasoline. Additional contingent tests for the oxyfuels may be required to further characterize other significant unexpected positive findings in the Alternative Tier 2 test battery. Other tests may also be required at the Tier 3 level, based on data from ongoing studies not related to the Alternative Tier 2 testing regimen, or to fill other existing data gaps of concern. Such additional tests could include evaluation of acute health symptoms in humans.

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DIFFERENTIAL GENE EXPRESSION OF ANTIOXIDANT ENZYMES IN MACROPHAGES EXPOSED TO CARBON PARTICLES ADSORBED WITH BENZO-[a]-PYRENE.

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Introduction

Airborne particulate matter can carry pollutants to the deep distal lung bypassing the respiratory defenses. In urban environments, up to 50% of the respirable particulate matter is carbonaceous. The major source of carbonaceous particles are cigarette smoke, diesel exhaust and incomplete combustion of fossil fuels (1-2). After deposition on bronchial and alveolar epithelium, the load of carbonaceous particles, if not exceeding the normal clearance capacity, are removed by the resident macrophages. Macrophages containing carbonaceous particles have been found in sputum of smokers (1). It has been hypothesized that the impairment of macrophage clearance of carbonaceous particles and the pollutants that they carry, mainly the polyaromatic hydrocarbons, may contribute to the lung-associated injury, presumably initiated by the inflammatory responses that may result in the release of cytokines inducing inflammatory cell recruitment, epithelial cell hypertrophy and hyperplasia (3). If the exposure is chronic, the persistence of these cellular functions can eventually lead to tumorigenesis. Unlike the other environmental pollutants such as ozone, sulfur dioxide and nitrogen dioxide, little is known about the mechanism of particle-induced lung injury. Since most pollutants produce injury that is oxidant-mediated and that cells respond to oxidant-stress by increasing the expression of antioxidant enzymes and stress response genes (4), we investigated whether exposure to carbonaceous particles induce expression of antioxidant enzymes and stress-response genes in macrophages and if so, how are they regulated. We have focused our studies on the stress-response gene product called heme oxygenase 1 (HO-1).

HO-1 is a microsomal membrane enzyme that catalyzes the first and rate-limiting reaction in heme catabolism, to yield equimolar quantities of biliverdin, iron and carbon monoxide. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. There are two isoforms of heme oxygenase, HO-1, the inducible form and HO-2, the constitutive form. Exposure of mammalian cells to cellular stresses such as heme, hypoxia, hyperoxia, lipopolysaccharide, cytokines, heavy metals, ultraviolet irradiation, gluthathione depletors, and hyperthermia have been shown to induce HO-1 gene expression (5-8). A common feature among the various inducers of HO-1 is that these agents, including heme, generate production of reactive oxygen species and/or modify gluthathione levels. This correlation and the observation that bilirubin, one of the end products of heme catabolism, functions as an antioxidant, has led to the hypothesis that HO-1 induction is part of a general response to oxidant stress and that this enzyme plays a protective role during such conditions (9).

In this study we hypothesized that carbon particles carrying the pollutant, Benzo-[a]-pyrene induce HO-1 gene expression in macrophages.We further examined the molecular regulation of HO-1 induction by carbon particles in macrophages.

Materials and Methods

Cell culture - A rat peritoneal macrophage cell line RAW 264.7 were obtained from American Tissue Cell Culture and were maintained in Dulbecco's Modified Eagles Medium supplemented with 10 % fetal bovine serum and gentamicin (50 µg/ml). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2/95% air. All experiments were performed with confluent cultures.

Model Carbon Particles -Two model carbon particles N339 and N339ox had preadsorbed on their surfaces a 0.75 monolayer of Benzo-[a]-Pyrene. To prevent agglomeration, the model particles were homogenized in DMEM media at 3000 *rpm* for 1 hour. Cells were exposed to N339+BAP and N339ox+BAP at [2 µg/ml] for up to 24 hours. Controls used for this study were model particles alone (N339 and N339ox) and also BAP alone. When stated, cells were pretreated with Actinomycin D (0.5 µg/ml), Cyclohexamide (1 µg/ml), Cytochalasin B (10 µg/ml) and N-Acetyl-cysteine (20 mM) for 1 h prior to carbon exposure.

RNA Extraction and Northern Blot Analysis - Total RNA was isolated by the STAT-60 RNAzol method with direct lysis of cells in RNAzol lysis buffer followed by chloroform extraction (10). Northern Blot analysis were performed as described

previously (11). Briefly, 10 µg aliquots of total RNA were fractionated on a 1% denaturing agarose gel, transferred to a nylon membrane by capillary action and cross-linked to the membrane by UV irradiation. The nylon membranes were incubated in hybridization buffer (1% bovine serum albumin, 7% SDS, 0.5M phosphate buffer, pH 7.0, 1.0 mM EDTA) containing ^{32}P labelled rat HO-1 cDNA (12) at 65°C for 24 hours. Nylon membranes were then washed twice in buffer A (0.5% bovine serum albumin, 5% SDS, 40 mM Phosphate buffer, pH 7.0, 1.0 mM EDTA) for 30 min. at 55°C followed by 4 washes in buffer B (1%SDS, 40mM Phosphate buffer pH 7.0, 1.0 mM EDTA) for 15 minutes at 55°C and exposed to X-OMAT film. To normalize for the amount of RNA in different samples or loading errors, blots were stripped and hybridized with radiolabelled oligonucleotide probe (5'-ACGGTATCTGATCGATCGTCTTCGAACC-3') complimentary to 18S rRNA. The values for the HO-1 mRNA transcript (1.8kb) were normalized to values for 18s rRNA obtained on the same blot. The HO-1 mRNA levels in the RAW exposed to model particles were expressed in densitometric absorbance units, normalized to control untreated samples, and expressed as fold induction relative to controls.

Western Blot Analyses - Cells were homogenized in lysis buffer (1% Nonidet P-40, 20 mM Tris, pH 8.0, 137.5 mM NaCl, 1 mM Na_2VO_4, 1 mM phenylmethylsulfonylfluoride, 10 µg aprotinin). Protein concentration of the lysates were determined by coomasie blue dye-binding assay (BioRad, NJ). An equal volume of 2X sample buffer (0.125M Tris-Hcl, pH 7.4, 4% SDS and 20% glycerol) was added to the sample and boiled for 5 minutes. Samples (100 µg) were electrophoresed in a 12% SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. The membranes were incubated with a rabbit polyclonal antibody against HO-1 (1:1000 dilution) for 1.5 hours following incubation with goat anti-rabbit IgG antibody for 1.5 hours. Signal development was carried out using an ECL detection kit (Amersham Corp, England).

DNA fragmentation assay - Genomic DNA were extracted as directed by the manufacturer for the Puregene™ DNA isolation kit (Gentra, NC). Twenty µg of DNA was load onto a 1.5% agarose gel in 1X Tris-Acetate buffer and subjected to electrophoresis.

RESULTS

Exposure to model particles adsorbed with Benzo-[a]-Pyrene (N339+BAP and N339ox+BAP) induces HO-1 gene expression in RAW cells -Cells were exposed to 2 µg/ml of both model particles, N339+BAP and N339ox+BAP for 1 h, 2 h, 4 h, 8 h and 24 h and HO-1 gene expression was examined by Northern blot analyses. There was a marked increase in the steady-state levels of HO-1 mRNA was observed at 4 h with the highest level of induction obtained after 8h of continuous exposure to N339+BAP and N339ox+BAP (Figure 1a). The accumulation of HO-1 mRNA levels correlated with increased HO-1 protein levels (Figure 1b).

Induction of HO-1 expression in RAW cells is dependent on phagocytosis of the model particles.
Cells exposed to N339+BAP for 8h demonstrated a 22 fold increase in HO-1 mRNA (Figure 2).To demonstrate that internalization of N339+BAP is required for HO-1 induction, we pretreated the RAW cells with 10 µg/ml of cytochalasin B, a potent inhibitor of phagocytosis for 1 h prior to exposure. Cytochalasin B pretreatment inhibited the N339+BAP induced HO-1 mRNA accumulation. Cytochalasin B treatment alone did not affect HO-1 mRNA levels.

HO-1 mRNA is dependent on gene transcription and de novo protein synthesis.
To further delineate the molecular basis for increased expression of HO-1 in response to N339+BAP, we examined whether HO-1 mRNA induction was dependent on gene transcription. Cells were pretreatment for 1 h with Actinomycin D, a potent inhibitor of RNA synthesis, prior to an 8 h exposure to N339+BAP. As shown in Figure 3, actinomycin D completely prevented N339+BAP induced HO-1 mRNA accumulation. We then determined whether HO-1 mRNA induction is dependent on *de novo* protein synthesis. Cells were pretreated with cycloheximide, a potent inhibitor of protein synthesis, for 1 h prior to an 8 h exposure to N339+BAP. Cyclohexamide also completely inhibited N339+BAP induced HO-1 mRNA expression.

Do reactive oxygen species (ROS) mediate N339+BAP induced HO-1 expression?
We determined whether phagocytosis of the model particle N339+BAP liberate ROS that may be responsible for inducing HO-1 expression. Pretreatment of cells with an antioxidant N-acetyl-cysteine (20 mM) 1 h prior to exposure to N339+BAP did not prevent the increase of HO-1 mRNA (Figure 4).

Phagocytosis of the model particle +BAP induces apoptosis in macrophages
Cells exposed to both model particles, N339+BAP and N339ox+BAP, for 24 h underwent apoptosis. Characteristic endonuclease generated DNA fragments of 160-180 bp are evident in RAW cells after 24h of continuous model particle exposure (Fig. 5)

Discussion

There has been much interest generated recently by reports demonstrating the induction of HO-1 gene expression by a variety of pro-oxidants (ultraviolet irradiation, hyperoxia and lipopolysaccharide) (13-16). This study demonstrates that exposure to model particles preadsorbed with Benzo-[a]-pyrene (N339+BAP and N339ox+BAP) also induces HO-1 gene expression in macrophages. The model particles selected for this study are well-defined carbon particles which are carbon blacks manufactured under conditions determined by the American Society for Testing Material (17). Since the surfaces of the environmental carbonaceous particles are heterogeneous, containing both oxidized and non-oxidized active sites, we decided to separate them and study their potential adverse effects. As controls for this study, the model particles alone (N339 and N339ox) as well as BAP separately did not induce HO-1 gene expression significantly (data not shown). The criteria for HO-1 induction requires both types of model particles to be a) adsorbed with BAP and b) internalized. The observation that we do not have a significant attenuation of HO-1 gene expression after pretreatment of the cells with NAC may suggest that HO-1 induction may not be modulated by reactive oxygen species. We have also examined other "prototypical" antioxidant enzymes that were exposed to these model particles. There were no significant changes in the mRNA levels of both CuZnSOD and MnSOD (data not shown).

Programmed cell death or apoptosis is a gene regulated process in which coordinated series of morphological changes such as nucleus and chromatin condensation, cell membrane blebing and fragmentation of cell into membrane-bound apoptotic bodies occur resulting in cell death. Removal of apoptotic bodies by phagocytosis by neighboring cells, in particular macrophages, occurs without initiating inflammation. Apoptosis is often a physiologic process, especially important during embryogenesis, organ atrophy and normal adult tissue turnover. However, accumulating evidence suggest that genotoxic and oxidant stress can induce cell death via apoptosis. Preliminary studies show that carbon particles can induce apoptosis in macrophages, and further studies are necessary to understand the regulation and function of carbon-induced apoptosis.

Future work will focus on delineating the transcriptional regulation and signal transduction pathways involved in the activation of the HO-1 gene by carbon particles. Furthermore, we will investigate whether carbon-induced HO-1 expression can serve to protect the macrophages from further cellular oxidant stress.

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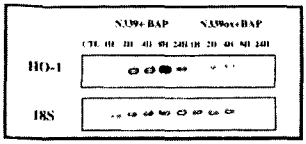

Fig. 1a. Northern blot analyses of HO-1 in RAW cells. Total RNA was extracted at the indicated times following continuous exposure to the model particles and analyzed for HO-1 mRNA expression. The ribosomal RNA 18s is shown as a normalization control.

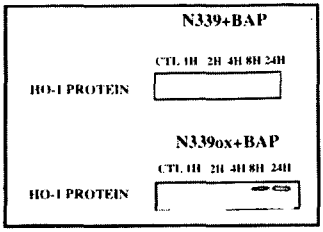

Fig. 1b. Western blot analyses of HO-1 protein in RAW cells. Total protein was extracted at the times indicated following continuous exposure to model particles and probed for HO-1 protein.

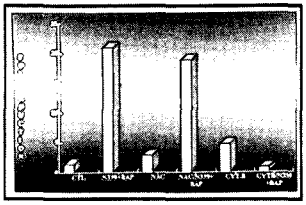

Fig. 2. Effects of NAC (20mM) and cytochalasin B (4ug/ml) on RAW cells exposed continuously to 8h N339+BAP. NAC pretreatment prior to N339+BAP exposure had no effect on HO-1 mRNA expression in RAW cells exposed to N339+BAP. Cytochalasin B pretreatment prior to exposure inhibited HO-1 mRNA expression. The results represent mean fold inducton by Northern blot analyses. 18s rRNA hybridization was used as a normalization control.

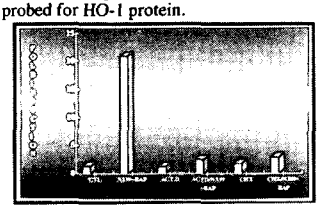

Fig. 3. Effects of Act. D (0.5ug/ml) and cyclohexamide (1ug/ml) on RAW cells exposed continuously to 8h N339+BAP. Act. D pretreatment prior to N339+BAP exposure prevented HO-1 mRNA expression in RAW cells exposed to N339+BAP. Cyclohexamide pretreatment prior to exposure also inhibited HO-1 mRNAexpression. The results represent mean fold induction by Northern blot analyses. 18s rRNA hybridization was used as a normalization control.

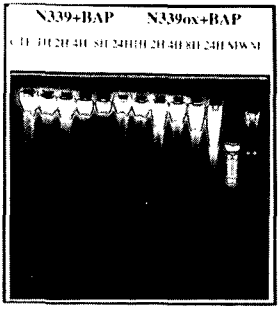

Fig. 4. Genomic DNA fragmentation gel assay of RAW cells. DNA (20 ug) was extracted at the times indicated following continuous exposure to model particles. Characteristic DNA fragments of 160-300 base pairs are evident at 24h of model particle exposure.